In this article, the area under the curve data were log_{10} transformed for normal distribution although they were already normally distributed. Though the article’s conclusion are unaltered, there are slight variations in the P-values upon applying the Bonferroni correction. The changes are marked in boldface.

The authors regret the errors.

ABSTRACT

Staphylococcus chromogenes TA showed significantly lower growth under iron-deprived conditions, and adding an iron supplement (lactoferrin or ferritin) resulted in no improvement in growth; in contrast, growth of S. chromogenes IM was significantly recovered with ferritin iron supplementation. Only Staphylococcus hominis strains originating from quarter milk were able to significantly utilize ferritin as an iron source to reverse the growth inhibition caused by chelating agent 2,2’-bipyridyl in varying degrees. Both S. chromogenes strains (IM and TA) and all S. hominis strains were unable to significantly use lactoferrin as an iron source for growth recovery.

RESULTS

Phenotypical Iron Test

Across all media, there was a significant strain effect (P = 0.0157), with S. aureus ATCC 25923 growing the best across all media [least squares means (LSM) of AUC = 7.22] but not significantly different from S. chromogenes IM (LSM = 6.37; P = 0.46) and S. hominis from TA (LSM = 5.79; P = 0.12; Table 2).

Over all strains, there was a considerable effect of medium (P < 0.0001), with the best growth observed on TSB (LSM = 8.14; Table 2). Growth was significantly better in media with ferritin (dTSBF; LSM = 5.37) as the iron source compared with dTSB (LSM = 3.76; Bonferroni-corrected P = 0.0001) and dTSBL (LSM = 4.59; Bonferroni-corrected P = 0.005). Additionally, lactoferrin as a supplement (dTSBL) made a significant difference in growth compared with dTSB (Bonferroni-corrected P = 0.027).

Thus, growth of the different strains was modified by the different media used, as indicated by the highly significant interaction term in the statistical model between medium and strain (P < 0.0001; Figure 2 and Table 2). Staphylococcus aureus showed overall comparable growth on the different media (Figure 2A). In contrast, for S. chromogenes IM, a significant reduction in maximum growth was observed in dTSB (Bon-
ferroni-corrected \( P < 0.0001 \); however, growth was significantly recovered when adding ferritin (dTSBF; Bonferroni-corrected \( P = 0.0006 \)) to the deferrated medium (Figure 2B; Supplemental Table S1, https://doi.org/10.6084/m9.figshare.24174876.v1, Reydams et al., 2023).

**DISCUSSION**

In this herd, *S. hominis* isolates were relatively highly genetically diverse, an overlapping RAPD type for *S. hominis* from RF and QM isolates was not found, and only *S. hominis* from QM were significantly better suited to utilize equine ferritin as an iron source than recombinant human lactoferrin. **Origin had an influence on growth in the different media for all *S. hominis* strains and both *S. chromogenes* strains.**

A total of 17 different NAS species were isolated and identified from the extensive quarter milk sampling on the herd performed specifically to find as many *S. hominis* isolates as possible from milk.

In accordance with a phenotypical iron test performed by Wuytack et al. (2019), *S. chromogenes* IM showed small differences in growth on different growth media, in contrast to *S. chromogenes* TA. While growth recovery with lactoferrin in iron-deprived media was not significant for *S. chromogenes* IM, when applying the Bonferroni correction (in contrast with ferritin), we observed this strain to utilize lactoferrin for growth recovery to some extent (\( P = 0.001 \) without Bonferroni correction, data not shown) when compared with *S. chromogenes* TA.

The ability of *S. hominis* from QM—but not from RF or TA—to grow to some degree in the presence of ferritin could **support the ability of *S. hominis* from QM to cause IMI**; however, none of the *S. hominis* isolates were able to significantly utilize lactoferrin as an iron source, suggesting that this species likely does not favor the mammary gland. This finding needs to be substantiated in further research by performing repeated monthly samplings in order to define a stringent IMI definition, and by measuring the SCC (Supré et al., 2011) to help determine the effects of *S. hominis* on udder health. Although *S. hominis* is usually considered part of the normal human skin microbiota, cows also harbor *S. hominis* in their gastrointestinal tract. Wuytack et al. (2020b) suggest that some NAS species originating from RF can cause IMI. However, our tested *S. hominis* strains from RF were unable to significantly use ferritin and lactoferrin for growth.
Figure 2. Overview of growth (optical density at 600 nm; OD$_{600}$) over 24 h in different media: tryptic soy broth (TSB), deferrated tryptic soy broth (dTSB), dTSB with ferritin from equine spleen (dTBSF), and dTSB with human recombinant lactoferrin (dTBSL) of (A) *Staphylococcus aureus* ATCC 25923, (B) *Staphylococcus chromogenes* “IM” (causing chronic IMI), (C) *S. chromogenes* “TA” (from teat apex of a heifer), (D) *Staphylococcus hominis* from quarter milk (QM), (E) *S. hominis* from rectal feces (RF), and (F) *S. hominis* from teat apices (TA). All experiments were performed in duplicate. Different letters within each figure (A–C) indicate significant differences when applying the Bonferroni correction between growth media within strains ($P \leq 0.05$).
recovery, making this niche an unlikely source of IMI, a hypothesis supported by the fact we did not find the same strains in the 3 different habitats.

REFERENCES